

AD \_\_\_\_\_

Award Number: DAMD17-98-1-8244

TITLE: Characterization of Tubulin Isoforms in Breast Cancer Cells

PRINCIPAL INVESTIGATOR: Asok Banerjee, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas Health  
Science Center at San Antonio  
San Antonio, Texas 78229-3900

REPORT DATE: May 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20010724 045

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> May 2001	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 May 00 - 30 Apr 01)	
<b>4. TITLE AND SUBTITLE</b> CHARACTERIZATION OF TUBULIN ISOFORMS IN BREAST CANCER CELLS			<b>5. FUNDING NUMBER</b>  DAMD17-98-1-8244	
<b>6. AUTHOR(S)</b> Asok Banerjee, Ph.D.			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  The University of Texas Health Science Center at San Antonio San Antonio, Texas 78229-3900 Email - asokb@worldnet.att.net				
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b> Report contains color graphics				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b>  Tubulin, the $\alpha\beta$ dimeric protein of microtubules, bind to different antitumor drugs which are routinely used for cancer chemotherapy. Both $\alpha$ - and $\beta$ -tubulin exist as 7-8 different isoforms which are expressed differently in different tissues, and also undergo various post-translational modifications including tyrosination-detyrosination, acetylation, poly-glutamylolation, polyglycylation and phosphorylation. It is not known whether breast cancer cells differ in the tubulin isoform level or their post-translational modifications. The long-term goal of this project will be to find new prognostic markers for an early detection of breast cancer and to develop alternative therapies against threast cancer. The primary goal is to study the expression of different forms of tubulin and their post-translational modifications in human breast cancer cells. Our previous results showed that paclitaxel resistant breast cancer cells express $\beta_{III}$ isoform selectively. Thus, it was felt necessary to make a full length cDNA of $\beta_{III}$ isoform for overexpression in breast cancer cells. Here we report the preparation of the cDNA and its overexpression in breast cancer cells. Stable MCF-7 cell lines overexpressing $\beta_{III}$ isoform are found to be more resistant to paclitaxel when compared with the wild-type MCF-7 cells.				
<b>14. SUBJECT TERMS</b> Breast Cancer, Tubulin, Maytansine, Paclitaxel, Podophyllotoxin Vinblastine, Colchicine, MCF-7, MDA-MB-231				<b>15. NUMBER OF PAGES</b> 16
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)  
Prescribed by ANSI Std. Z39-18  
298-102

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

\_\_\_\_ Where copyrighted material is quoted, permission has been obtained to use such material.

\_\_\_\_ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

\_\_\_\_ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

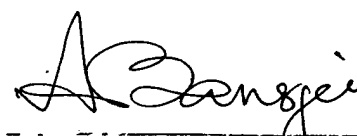
\_\_\_\_ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

\_\_\_\_ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

\_\_\_\_ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

\_\_\_\_ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

  
PI - Signature

May 30, 2001  
Date

## TABLE OF CONTENTS

FRONT COVER.....	1
STANDARD FORM (SF) 298, REPORT DOCUMENTATION PAGE.....	2
FOREWORD .....	3
TABLE OF CONTENTS.....	4
INTRODUCTION.....	5
BODY.....	6-12
KEY RESEARCH ACCOMPLISHMENTS.....	13
REPORTABLE OUTCOMES AND CONCLUSIONS .....	14
APPENDICES .....	15

## Introduction

Tubulin, the heterodimeric  $\alpha\beta$  subunit of microtubules, exist as multiple isoforms whose expression pattern differ from one tissue to the other (1-4). In mammalian system, there are about 5-7 different isoforms of  $\alpha$ - and  $\beta$ - tubulin (5-13). Both  $\alpha$ - and  $\beta$ -tubulin also undergo a variety of post-translational modifications (14-25);  $\alpha$ -tubulin undergoes tyrosination-detyrosination at the C-terminus and acetylation at Lys<sup>40</sup> (20,21);  $\beta_{III}$ -tubulin undergoes phosphorylation at a Ser residue (23-25); both  $\alpha$ - and  $\beta$ -tubulin also undergo polyglutamylation and polyglycylation, in which glutamyl or glycyl units are attached as side chains through the  $\gamma$ -carboxyl of a Glu residue near the carboxy terminal (15-19). We have previously prepared monoclonal antibodies to different tubulin isoforms, and also purified some of the isoforms from bovine brain (26-40). Isotypically pure different tubulin dimers have been found to differ in their assembly, conformation, and binding to antitumor drugs (26-40).

The primary aim of this project is to study the expression of different tubulin isoforms and their post-translational modifications in breast cancer cells. The idea is to see whether there is any difference in breast cancer tubulin that can be utilized to discover prognostic markers or novel targets for the treatment of breast cancer. The project is on its final year, and has so far opened up new lines of research that was not present in the original proposal.

### **Expression of tubulin isoforms in human breast cancer cells resistant to paclitaxel:**

#### **Preparation of drug-resistant Breast Cancer Cells:**

It has been reported that certain tubulin isoforms get expressed when cancer cells become resistant to anticancer drugs. To study the tubulin isoforms we prepared breast cancer cells resistant to antimitotic drugs. The cell lines were prepared by initially growing breast cancer cell lines MCF-7 and MDA-MB-231 in the presence of 1 nM of colchicine, podophyllotoxin, vinblastine or paclitaxel. Verapamil was kept in the growth medium to exclude multidrug-resistant cells. The drug concentration was gradually increased by 1.5 times. After 3-4 months of selection, two drug-resistant lines **MCF7/PTX20** (resistant

to paclitaxel) and **MDA-MB-231/POD60** (resistant to podophyllotoxin) were obtained.

### **Immunoblot analysis of $\beta$ -tubulin isoforms in drug-resistant breast cancer cells:**

The drug-resistant breast cancer cells were grown to confluency in T-150 culture flasks. The cells were trypsinized and harvested. The cell extract was mixed with equal volume of 2X Laemmli sample buffer, boiled for 5 min, and was analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting using monoclonal antibodies to  $\beta_{II}$ ,  $\beta_{III}$ , and  $\beta_{IV}$ . The paclitaxel-resistant MCF-7 cells contains much higher amounts of  $\beta_{II}$  and  $\beta_{III}$  as compared to the drug-sensitive wild type cells. The amount of  $\beta_{IV}$  was marginally increased in the resistant cells. On the other hand, podophyllotoxin-resistant cells exhibited a decrease in the content of all three isoforms  $\beta_{II}$ ,  $\beta_{III}$ , and  $\beta_{IV}$ . Since no antibody was available, it was not possible to see the status of the other  $\beta$ -tubulin isoforms by immunoblotting. **At this point it is not clear why the level of some of the isoforms gets elevated while that of others decrease. It may be possible that the cells can identify those isoforms that have the lowest interaction with the drug, and specifically overexpress those isoforms, while the isoforms that have the highest affinity for the drug get lower expression.**

### **Preparation of cDNA constructs for the expression of GFP-tagged $\beta_{III}$ tubulin in human cancer cells**

In an effort to overexpress individual tubulin isoforms, I have been working on the cloning of full length cDNA specific for individual tubulin isoforms. By using primers specific for  $\beta_{III}$  tubulin, I have prepared full length 1353 bp cDNA from total RNA isolated from MCF-7 cells by RT-PCR using AMV reverse transcriptase and taq DNA polymerase. The product showed a single 1353 bp band in 1.5% agarose gel.

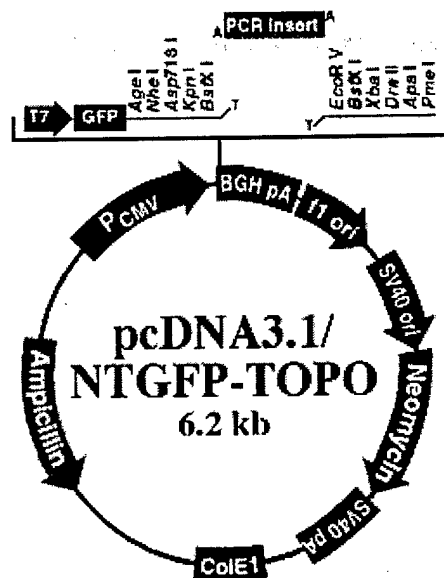
Primers for cloning full-length cDNA for  $\beta_{III}$  tubulin (Human):

Forward: 5'- ATG CCG GAG ATC GTG CAC ATC -3' (+1- 21)

Reverse: 5'- TCA CTT GGG GCC CTG GGC CTC-3' (+1330-1353)

The PCR product was inserted in the following mammalian expression vector PC DNA 3.1 GFPNT TOPO (obtained from Invitrogen ) at the multiple

cloning site in a 5 minute ligation process and was used to transform *E. coli* *Top 10* competent cells.



The vector has an **ampicillin resistant gene** for the selection of bacteria and a **neomycin resistant gene** for the selection of stable transfectants for mammalian expression. The transformed mixture was plated on an LB agar plate containing ampicillin and was incubated overnight at 37°C. About 10 -15 colonies were picked, grown in LB broth overnight, and the plasmids were prepared by a miniprep procedure using a Qiagen kit. The plasmids were tested for the insert size as well as the orientation by restriction digestion followed by agarose gel electrophoresis (since the vector can integrate the DNA in both orientation). The insert size was checked by a double digestion using *Kpn* I and *EcoR* V. The orientation was checked by a double digestion with *Kpn* I and *Bam*H I. Since the  $\beta_{III}$  cDNA sequence (1350 bp) has a *Bam*H I site at the base 1032, the plasmid with the right orientation yields a fragment of 1050 bp while the plasmid with a wrong orientation yields a fragment of 350 bp. After selecting the correct plasmid the sequence was confirmed by DNA sequencing. The sequence matched almost perfectly with that of the published sequence of human  $\beta_{III}$  tubulin.

### **Overexpression of GFP-tagged $\beta_{III}$ tubulin in human breast and ovarian cancer cells**

The  $\beta_{III}$  plasmid was constructed as above at the 3' end of a GFP open reading frame under the influence of a CMV promoter so that GFP is tagged at N-terminal of  $\beta_{III}$  tubulin. Human breast cancer cells (MCF-7) and the ovarian cancer cells (SK-OV-3) were transfected with the GFPNT- $\beta_{III}$  plasmid using the Lipofectamine Plus reagent from GIBCO BRL. The cells were plated on a 12-well plate on the previous day. The transfection was carried out in DMEM without fetal bovine serum for 5 h at 37°C in a humidified CO<sub>2</sub> incubator. After 5 h, serum was added to the cells. **For transient transfection**, the cells were plated over tissue culture treated glass cover slips. The samples were examined at 24 h, 48h, and 72h, under a fluorescence microscope using a FITC filter set for visualization of green fluorescence.

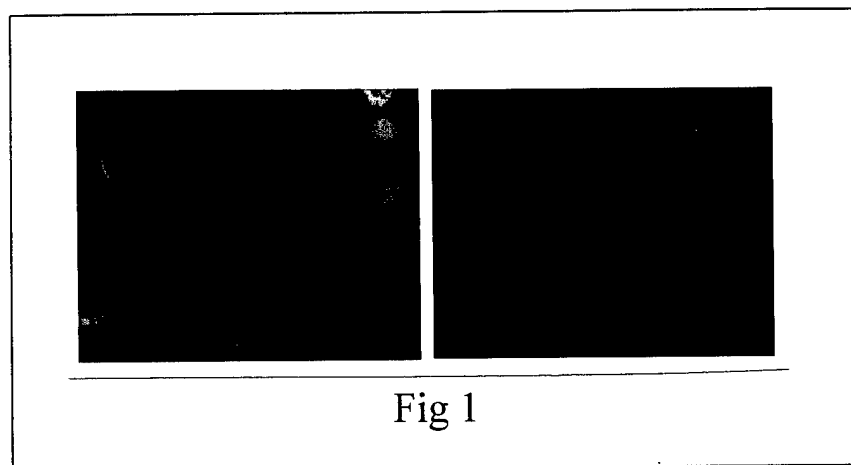


Fig 1

**For stable transfection**, after 24h of transfection the cells were maintained in the complete medium containing 0.5 mg/ml G 418 (neomycin). After 2-3 weeks, when the colonies started showing up, the cells were given passages to bigger flasks. Fig 1 shows the fluorescence microscopic photographs of stable SK-OV-3 cells overexpressing GFP-tagged  $\beta_{III}$  tubulin (green, left). The photograph on the right shows the immuno-fluorescence staining of the same cells treated with a monoclonal antibody to tyrosinated  $\alpha$ -tubulin (AYN 6D.10) followed by an incubation with Cy3-labeled secondary antibody (red, right).



### **The effect of overexpression of $\beta_{III}$ tubulin on the paclitaxel sensitivity of SK-OV-3 ovarian cancer cells:**

In a preliminary experiment, we have compared the PTX sensitivity of the wild type SK-OV-3 ovarian cancer cell line and the cells overexpressing GFP-tagged  $\beta_{III}$  tubulin. We find that the  $IC_{50}$  value of paclitaxel is increased significantly in cells overexpressing  $\beta_{III}$ . The experiment was carried out on a mass culture, where the expression of  $\beta_{III}$  are not same for all the cells. The experiment need to be repeated with selected clones. These data indicate that an increase in the expression of tubulin may reduce the drug sensitivity. This will be tested in MCF-7 breast cancer cells.

### **Post-translational modifications in breast cancer tubulin:**

Efforts were initiated to study the post-translational modifications in tubulin from breast cancer cells. In a preliminary experiment, cell extracts from MCF7 and MDA-MB-231 cells were tested for the acetylation and tyrosination status in tubulin. When compared with bovine brain tubulin, both the acetylation and the tyrosination status in breast cancer tubulin seems to be elevated. Since the data are too preliminary and are without the right control cells, the experiments will be repeated. At present, the tubulin is being purified from the breast cancer cells and the purified tubulin will be tested for its post-translational polyglutamylolation and polyglycylation by MALDI TOF mass spectrometry.

### **MALDI TOF mass spectrometry of bovine brain tubulin:**

Before doing the MALDI TOF analysis on purified tubulin from the breast cancer cells, we tested the procedure on the tubulin purified from bovine brain. Since it is easily available, we can isolate milligram quantities of purified tubulin.

### **Separation of $\alpha$ -tubulin fractions:**

Bovine brain tubulin contains two  $\alpha$ -tubulin isoforms,  $\alpha_{1/2}$  and  $\alpha_4$ . Using an anti- $\alpha$ -tubulin immunuaffinity column we have previously fractionated bovine brain tubulin into three functionally active forms, fractions A, B, and C (40). Fraction A mainly contains non-tyrosinated forms of  $\alpha_{1/2}$  including  $\Delta 2$  tubulin. Fraction B is a mixture of the non-tyrosinated forms of  $\alpha_{1/2}$  and  $\alpha_4$ . Fraction C is essentially the tyrosinated form of  $\alpha_{1/2}$ .

Mass spectrometric studies revealed that the fraction C ( $\alpha^{\text{Tyr}}1/2$ ) is polyglutamylated with 1-4 Glu residues, tetraglutamylated form being the predominate one. On the other hand, the fraction A is found to contain post-translationally added 1-3 glycine residues.

### **Key Research Accomplishments**

- We have studied the expression of tubulin isoforms in breast cancer cell lines by immunoblotting and RT-PCR analysis.
- Paclitaxel resistant MCF-7/PTX20 cells express increased amounts of  $\beta_{II}$  ,  $\beta_{III}$  but not  $\beta_{IV}$ .
- Podophyllotoxin resistant MDA-MB-231 POD60 cells express lower amounts of  $\beta_{II}$  ,  $\beta_{III}$ , and  $\beta_{IV}$ .
- We have prepared the full-length cDNA for  $\beta_{III}$  -tubulin from MCF-7 breast cancer cells.
- We have prepared GFP-tagged  $\beta_{III}$  -tubulin construct in the vector PC DNA 3.1 Topo.
- We have overexpressed GFP-tagged  $\beta_{III}$  -tubulin in MCF-7 breast cancer cells and obtained stable cell lines.
- MCF-7 cells overexpressing  $\beta_{III}$  -tubulin are more resistant to paclitaxel than the wild-type MCF-7 cells.
- These results support the hypothesis that tubulin isoform level in a cell line may determine the drug sensitivity. This is a major finding.
- We have performed preliminary MALDI TOF mass spectrometric studies for the identification of post-translational modifications in bovine brain tubulin. We have discovered polyglycylation in brain tubulin. Similar studies will be performed on the tubulin from breast cancer cells.

## **Reportable Outcomes**

1. We have overexpressed GFP-tagged  $\beta_{III}$ -tubulin in MCF-7 breast cancer cells and obtained stable cell lines.
2. We find that the MCF-7 cells overexpressing  $\beta_{III}$ -tubulin are more resistant to paclitaxel than the wild-type MCF-7 cells.
3. Our results support the hypothesis that tubulin isoform level in a cell line may determine the drug sensitivity. This is a major finding.
4. We have performed preliminary MALDI TOF mass spectrometric studies for the identification of post-translational modifications in bovine brain tubulin. We have discovered polyglycylation in brain tubulin.

## **Publication**

Four manuscripts will soon be submitted for publication:

1. Banerjee, A. (2001) Assembly of alpha-tubulin isoforms with different post-translational modifications.  
Manuscript to be submitted to J. Biol. Chem.
2. Banerjee, A. (2001) MALDI TOF mass spectrometric evidence for the presence of post-translational glycylation in bovine brain tubulin.  
Manuscript to be submitted to J. Biol. Chem.
3. Banerjee, A. (2001) Expression of  $\beta$ -tubulin isoforms in podophyllotoxin-resistant human breast cancer cells.  
Manuscript to be submitted to J. Biol. Chem.
4. Banerjee, A. (2001) Overexpression of  $\beta_{III}$  tubulin in MCF-7 breast cancer cells increases resistance to paclitaxel.  
Manuscript to be submitted to J. Biol. Chem.

## References

1. Olmsted, J. B. and Borisy, G. G. (1973) Microtubules, *Annu. Rev. Biochem.*, **42**, 507-540
2. Dustin, P. (1978) *Microtubules*, Springer-Verlag, Berlin and New York.
3. Roberts, K and Hyams, J. S. (ed.) (1979) Biochemistry of tubulin, in *Microtubules*, Academic Press, New York.
4. Avila, J. (1990) *Microtubule Proteins*, CRC press, Florida.
5. Villasante, A., Wang, D., Dobner, P., Dolph, P., Lewis, S. A., and Cowan, N. J. (1986) Six mouse  $\alpha$ -tubulin m-RNAs encode five distinct isotypes: Testis-specific expression of two sister genes, *Mol. Cell. Biol.* **6**, 2409-2419.
6. Sullivan, K. F. and Cleveland, D. W. (1986) Identification of conserved isotype-defining variable region sequences for four vertebrate  $\beta$ -tubulin polypeptides, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4327-4331.
7. Wang, D., Villasante, A., Lewis, S.A. and Cowan, N.J. (1986) The mammalian  $\beta$ -tubulin repertoire: Hematopoietic expression of a novel, heterologous,  $\beta$ -tubulin isotype, *J. Cell Biol.* **103**, 1903-1910
8. Murphy, et al (1987) The sequence and expression of the divergent  $\beta$ -tubulin in chicken erythrocytes, *J. Biol Chem.* **262**, 14305-14312
9. Monteiro, M. J. and Cleveland, D. W. (1988) Sequence of chicken C $\beta$ 7 tubulin: Analysis of a complete set of vertebrate  $\beta$ -tubulin isotypes, *J. Mol. Biol.* **199**, 439-446.
10. Lee, G.N., Cowan, N.J., and Kirschner, M.W. (1988) *Science* **239**, 285-288
11. Lewis, S.A., Wang, D., and Cowan, N.J. (1988) Microtubule-associated protein MAP2 shares microtubule binding motif with tau protein, *Science* **242**, 936-939
12. Sullivan, K. (1988) Structure and utilization of tubulin isotypes, *Ann. Rev. Cell Biol.* **4**, 687-716.

13. Stanchi, F, et al. (2000) TUBA8: A new tissue-specific isoform of  $\alpha$ -tubulin that is highly conserved in human and mouse.  
*Biochem. Biophys. Res. Commun.* **270**, 1111-1118
14. Barra, H. S., Arce, C. A., Rodriguez, J. A., and Caputto, R. (1974)  
*Biochem. Biophys. Res. Commun.* **60**, 1384-1390
15. Mary, J., Redeker, V., La, Caer, J.-P., Promé, J.-C., and Rossier, J. (1994)  
*FEBS Lett.* **353**, 89-94
16. Rüdiger, M., Plessman, U., Rüdiger, A.-H., and Weber, K. (1995)  
*FEBS Lett.* **364**, 147-151
17. Ersfeld, K. Wehland, J, Plessman, U., Dodemont, H., Gerke, V., and Weber, K (1993) *J. Cell Biol.* **120**, 725-732
18. Paturle-Lafanechère, Eddé, B., Denoulet, P., Van Dorsselaer, A., Mazarguil, H., La, Caer, J.-P, Wehland, H., and Job, D (1991)  
*Biochemistry*, **30**, 10523-10528
19. Rüdiger, M., Wehland, J., and Weber, K. (1994)  
*Eur. J. Biochem.* **220**, 309-320
20. L' Hernault, S.W., and Rosenbaum, J. L. (1983) *J. Cell Biol.* **97**, 258-263
21. L' Hernault, S. W., and Rosenbaum, J. L. (1985)  
*Biochemistry*, **24**, 463- 478
22. Eddé, B., Rossier, J., Le Caer, J.-P., Desbruyeres, E., Gros, F., and Denoulet, P. (1990) *Science*, **247**, 83-84
23. Redeker, V., Levilliers, N., Schmitter, J.-M, Le Caer, J.-P., Rossier, J., Adoutte, A., and Bré, M.-H. (1994) *Science*, **266**, 1688-1691
24. Alexander, J. E., Hunt, D. F., Lee, M. K., Shabanowitz, J., Michel, H., Berlin, S. C., Mac Donald, T. M., Sundberg, R. J., Rebhun, L. I., and Frankfurter, A. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4685-4689
25. Eddé, Jeantet, C., and Gros, F. (1981)  
*Biochem. Biophys. Res. Commun.* **103**, 1035-1043.
26. Banerjee, A., Barnes, L.D., and Luduena, R.F. (1987) The role of the B-ring of colchicine in the stability of the colchicine-tubulin complex,  
*Biochim. Biophys. Acta* **913**, 138-144
27. Banerjee, A. and Luduena, R.F. (1987) Kinetics of association and dissociation colchicine-tubulin complex from brain and renal tubulin: Evidence for the exis of multiple isotypes of tubulin in brain with differential affinity to tubulin,  
*FEBS Lett.* **219**, 103-107

28. Banerjee, A., Roach, M.C., Wall, K.A., Lopata, M.A., Cleveland, D.W., and Luduena, R.F. (1988) A monoclonal antibody against the type II isotype of  $\beta$ -tubulin: Preparation of isotypically altered tubulin, *J. Biol. Chem.* **263**, 3029-3034.
29. Banerjee, A., Roach, M.C., Trcka, P.P. and Luduena, R.F. (1990) Increased microtubule assembly in bovine brain tubulin lacking the type III isotype of  $\beta$ -tubulin, *J. Biol. Chem.* **265**, 1794-1799.
30. Banerjee, A., Roach, M.C., Trcka, P., and Luduena, R.F. (1992) Preparation of a monoclonal antibody specific for the class IV isotype of  $\beta$ -tubulin: Purification and assembly of  $\alpha\beta_{II}$ ,  $\alpha\beta_{III}$ , and  $\alpha\beta_{IV}$  tubulin dimers from bovine brain, *J. Biol. Chem.* **267**, 5625-5630.
31. Banerjee, A. and Luduena, R.F. (1991) Distinct colchicine binding kinetics of bovine brain tubulin lacking the type III isotype of  $\beta$ -tubulin, *J. Biol. Chem.* **266**, 1689-1691.
32. Banerjee, A., and Luduena, R.F. (1992) Kinetics of colchicine binding to purified  $\beta$ -tubulin isotypes from bovine brain, *J. Biol. Chem.*, **267**, 13335-13339.
33. Banerjee, A., and Luduena, R.F. (1991) Assembly of isotypically pure tubulin in the presence of taxol, *J. Cell Biol.* **115**, p337a.
34. Panda, D., Miller, H. P., Banerjee, A., Luduena, R. F., and Wilson, L. (1994) Microtubule dynamics *in vitro* are regulated by the tubulin isotype composition. *Proc. Natl. Acad. Sci. USA* **91**, 11358-11362.
35. Banerjee, A., D'Hoore, A., and Engelborghs, Y. (1994) Interaction of desacetamidocolchicine, a fast-binding analogue of colchicine with isotypically pure tubulin dimers  $\alpha\beta_{II}$ ,  $\alpha\beta_{III}$ , and  $\alpha\beta_{IV}$ . *J. Biol. Chem.* **269**, 10324-10329.
36. Banerjee, A., Engelborghs, Y., D'Hoore, A., and Fitzgerald, T. J. (1997) Interaction of a bicyclic analog of colchicine with purified  $\beta$ -tubulin isoforms from bovine brain. *Eur. J. Biochem.* **246**, 420- 424.
37. Banerjee, A. (1997) Differential effects of colchicine and its B-ring-modified analog MTPT on the assembly-independent GTPase activity of purified  $\beta$ -tubulin isoforms from bovine brain. *Biochem. Biophys. Res. Commun.* **231**, 698-700.
38. Banerjee, A. And Kasmala, L.T. (1998) Differential assembly kinetics of  $\alpha$ -tubulin isoforms in the presence of paclitaxel.

- Biochem. Biophys. Res. Commun.* **245**, 349-351.
39. Banerjee, A., Kasmala, L. T., Hamel, E., Sun, L., and Lee, K.-H. (1999) Interaction of novel thiocolchicine analogs with the tubulin isoforms from bovine brain. *Biochem. Biophys. Res. Commun.* **254**, 334-337.
40. Banerjee, A. (1999) A monoclonal antibody to  $\alpha$ -tubulin: Purification of functionally active  $\alpha$ -tubulin isoforms. *Biochemistry*, **38**, 5438-5446